SOME MICROSCOPIC STUDIES OF THE KERATINIZATION OF HUMAN HAIR*

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Theories concerning the process of keratinization in human hair have arisen from morphologic observations. These morphologic concepts invariably link the process of keratinization with readily observable cytologic changes occurring progressively in a proximodistal direction in the lower one-third of the hair follicle. The final histologic change, the attainment of optical homogeneity by hair cortex cells, is regarded by most histologists as the final stage in the keratinization process. This change regularly occurs at a level about one-third of the distance from the tip of the hair papilla to the skin surface (1). The observations of Giroud and Bulliard (2) concerning the abrupt transformation of sulfhydryl groups into disulfide linkages at or near this level have added weight to the morphological concepts.

In 1949, however, Mercer (3) performed a straightforward series of physicochemical experiments on plucked human hairs, and he thereby demonstrated that the formation of fully keratinized hair was a process occupying the entire length of the hair root enclosed by the inner root sheath, rather than the lower one-third of the hair root. Mercer treated plucked intact human hair roots with a variety of physical and chemical agents (50 per cent urea solution, enzymes, acids, alkali, and heat), recording induced changes in birefringence and x-ray diffraction patterns. The observations led Mercer to distinguish several zones of varying stability of molecular structure in the forming hair cortex. His technics were necessarily destructive of the soft tissues of the hair bulb where the earliest stages of hair formation take place. Hence a re-examination of the entire hair root by selective staining methods seemed warranted in order to correlate cytological structures and their staining properties with the protein transformations in the forming hair cortex.

MATERIALS AND METHODS

For the purpose of correlation with Mercer's studies, plucked human hairs from six living humans were obtained. The hair roots from growing hairs (identified by the retention of sheaths about the roots) were mounted on slides in immersion oil.

For the remainder of the studies hair-bearing skin from human scalp was obtained at autopsy. For the demonstration of double refraction, tissue was frozen unfixed and stored at −30°C. prior to sectioning at −20°C. in a cryostat (after the method described by Coons, Leduc, and Kaplan (4)). Specimens were mounted directly in glycerogel for observation with a Leitz polarizing microscope. Retardation measurements were made under oil immersion with a Berek compensator. Birefringence ($n_{\parallel} - n_{\perp}$) was calculated from

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the equation $(n_{\parallel} - n_{\perp}) = \frac{\Delta\lambda}{d}$ where $\Delta\lambda$ is retardation and d is thickness of specimen as measured with the micrometer focusing screw.

For the histochemical demonstration of protein-bound sulfhydryl groups the method of Barrnett (5) was employed. The same technic was utilized on sections previously exposed to a 50 per cent solution of yellow ammonium sulfide for two hours at 50°C. in order to reduce disulfide linkages to demonstrable thiol groups (6). Ribonucleic acid was demonstrated by the technic of Deane (7), and staining was carried out with 5×10^{-4} M methylene blue or 5×10^{-5} M toluidine blue buffered to pH 5.0 and stained at 25°C. for 24 hours.

For determination of the acid and basic dye binding properties of the hair, ethanol-fixed paraffin-embedded tissues were sectioned and stained with an acid dye (orange G) and a basic dye (methylene blue) by the method described by Singer and Morrison (8). Each dye* was employed separately in a molar concentration of 5×10^{-4} in a series of buffered staining baths whose pH varied in the range 3.0 to 8.0; buffer solutions of 0.01 ionic strength were used and staining was carried out for 24 hours at 25°C. in 1500 cc. of dye solution. After staining, the sections were washed briefly in distilled water, dehydrated in tertiary butyl alcohol, cleared with xylol and mounted in Permount. Dye-binding capacity was determined by microscopic observation of colors in specified histological structures at each pH employed.

OBSERVATIONS

For descriptive purposes terminology adapted from Mercer will be used. Mercer designated five levels (A through E) occurring proximodistally from the base of the follicle at 0, 500, 1200, 1800, and 2000 μ respectively. Mercer identified zone AB as the isotropic bulb region, level B being indicated as the point at which fibrillation occurs. Zone BC he called the zone of unstabilized fibrils to indicate the presence of oriented structures weakly held together by weak bonds. CDE was named the consolidation zone, a region of progressive hardening of keratin in which the final hardening of hair occurs. Histologically this zone is optically homogeneous and hence has been regarded as fully keratinized (1).

In the present study an histologic correlation of Mercer's zones was achieved by comparison of intact plucked human hairs and microscopic sections of human hair. The only discrepancy with Mercer's findings arose with the observation that fibrillation occurs throughout the upper half of Mercer's zone AB, and in consequence this region will be referred to as the fibrillation zone (see diagram in Fig. 6).

Birefringence of precortical intracellular fibrillar structures was observed to appear first at a level adjacent to the upper pole of the papilla (Fig. 1). This finding locates the earliest development of intracellular fibrillae at a lower level than that described by Mercer and by Schmidt (9).

Measurements of the birefringence of intact plucked hairs were in agreement with the findings of Mercer, suggesting that the final value of birefringence (0.011) is rapidly attained in the zone of unstabilized fibrils. However, measurements made on longitudinal sections of hair roots reveal a more gradual rise in double refraction throughout the zones of fibrillation, unstable fibrils, and consolidation, the maximum value of 0.011 being attained only in fully hardened

* The orange G sample was 86 per cent pure and the methylene blue sample 99 per cent pure.

hair (Fig. 2). Further studies with reference to diameter of cortex and volume of fibrils would be required to explain satisfactorily the differences observed between measurements of birefringence in intact and sectioned hair.

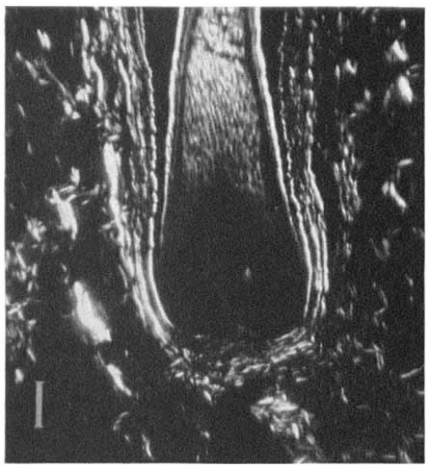

FIG. 1. Development of birefringence in human hair bulb. Birefringent fibrillar structures will be observed adjacent to the upper pole of the hair papilla in the developing hair cortex. The birefringence of the internal root sheath appears at a lower level than that of the precortical structures. Unfixed tissue section mounted in glycerogel photographed in polarized light with prisms crossed. ×100.

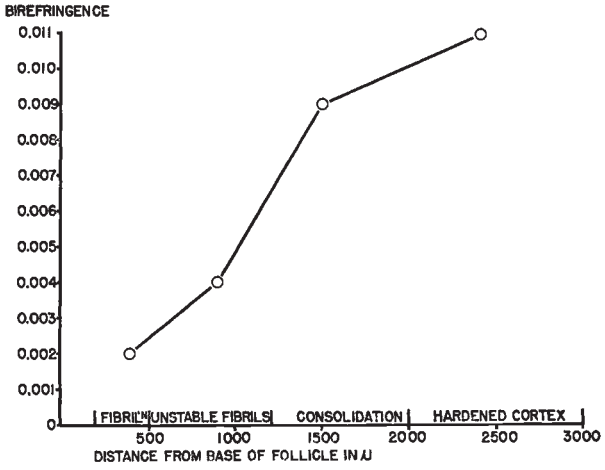

FIG. 2. Graphic presentation of birefringence measurements at selected levels in the developing cortex of human hair (see text). Measurements made with a Berek compensator on microscopic sections of unfixed tissue mounted in glycerogel.

Barrnett's sulfhydryl method produces a moderately intense reaction in the lower bulb, becoming maximally intense in the zone of fibrillation and persisting thus throughout the zone of unstabilized fibrils. Beyond this region the reaction

abruptly becomes negative (Fig. 3). This distribution concurs with Barrnett's observations in rat hair (10) and is in accordance with the illustrated findings of Giroud and Bulliard (2), who used the alkaline nitroprusside reaction on horse skin.

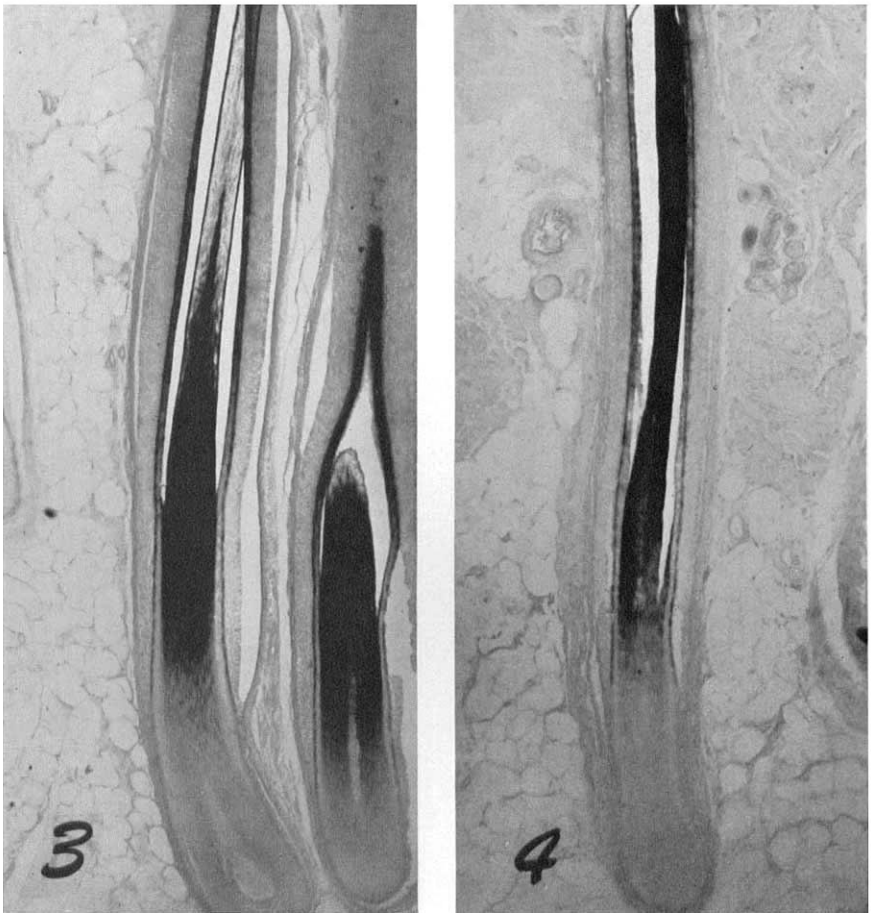

FIG. 3. Protein-bound sulfhydryl groups in the fibrillation zone and region of unstable fibrils. Note lack of reaction in the consolidation zone. Barrnett's sulfhydryl technic. ×35.

FIG. 4. Disulfide groups demonstrated in consolidation zone of developing hair cortex. The presence of reactive groups below the region of consolidation cannot be ascribed to S-S linkages because reactive thiol groups appear in these regions (cf. Fig. 3). Barrnett's method applied to specimen previously reduced in ammonium sulfide solution. ×35.

Treatment of sections with ammonium sulfide solution to render disulfide linkages stainable with the Barrnett reagents reveals disulfide groups to be present in considerable concentration in the consolidation zone as well as the fully hardened cortex (Fig. 4). (Identification of disulfide groups in regions shown to be rich in sulfhydryl groups is not possible with this method.)

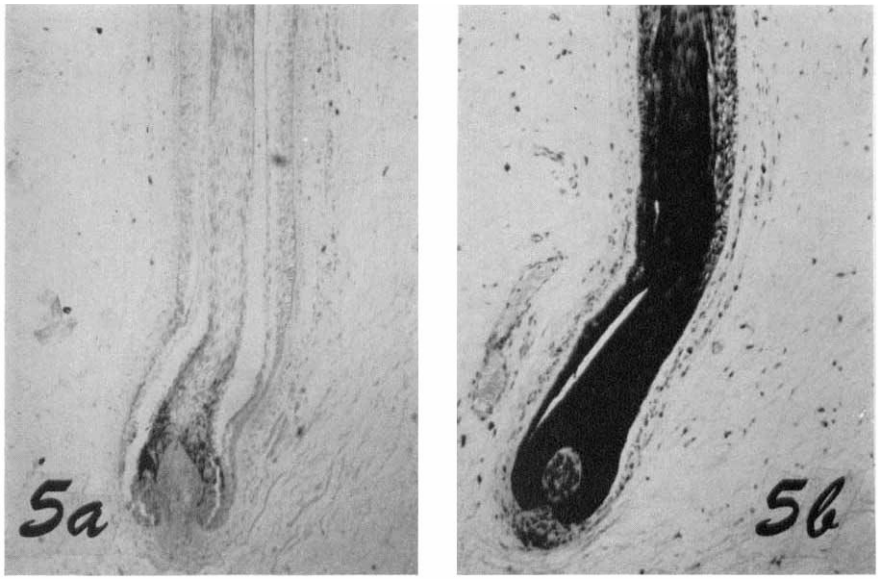

FIG. 5. The figure on the left (5a) reveals staining of section exposed to ribonuclease. The basophilia observed in the control section (5b) not present in the specimen at the left is attributable to ribonucleic acid. Its distribution will be observed in the fibrillation zone and lower part of the unstable fibrous region. ×35.

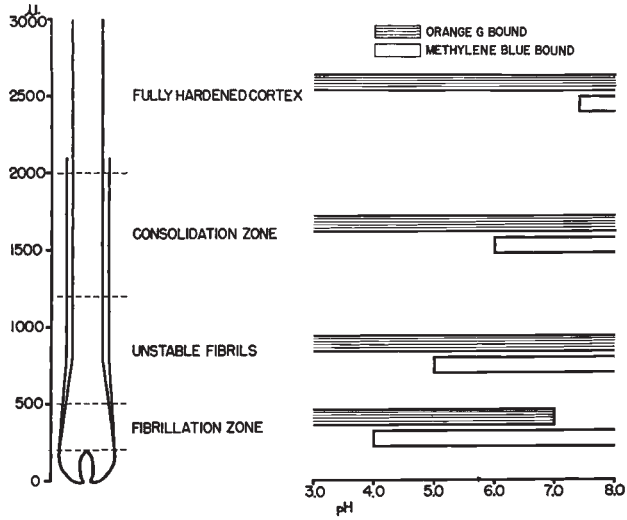

FIG. 6. Chart summarizes the capacity of fibrous structures of the developing hair cortex to bind the acid and basic dyes, orange G and methylene blue. The diagram of the hair follicle and root to the left indicates the zones of keratinization referred to in the text with respect to their distance in micra from the base of the follicle. The extent of the internal root sheath is indicated by the most laterally-placed lines.

Ribonucleic acid appears in the cytoplasm of undifferentiated cells of the hair bulb, reaching maximal concentration in the fibrillation zone and gradually disappearing in the region of unstable fibrils (Fig. 5). These observations are in accord with the observations of Hardy (11) on mouse hair. It is further to be noted that unidentified basophilic substances in the unstable fibril zone are not removed by the specific enzymatic activity of ribonuclease and therefore may not be regarded as ribonucleotides.

The capacity for biological structures to bind simple acid or basic dyes has not been thoroughly explored in connection with the forming hair. In the present study the binding of the acid dye, orange G, and the basic dye, methylene blue, was observed at several pH intervals within the range 3.0 to 8.0. The principal findings are summarized in Fig. 6, where the dye-binding properties of the fibrous structures of the hair-forming cortex are recorded.

It will be seen that staining of fibrillar structures of the fibrillation zone with acid dye occurred from pH 3.0 to pH 7.0 but was not present at pH 8.0. The fibrils of the unstabilized fibrous zone and the homogeneous structures of the consolidation zone bind acid dye throughout the range employed. On the other hand, a gradual decrease in basic dye binding capacity is observed in the progressive sequence of keratinization; fibrils of the fibrillation zone bind basic dye from pH 4.0 upward, whereas the fully hardened cortex does not bind basic dye below pH 7.4.

DISCUSSION

Auber (1) points out in his extensive study of the histology of hair formation that mitotic activity in precortical cells ceases and cytoplasmic volume increases at a level in the hair bulb which coincides with the beginning of the fibrillation zone defined in the present study. In this zone there appear birefringent fibrillae in cells shown to be rich in ribonucleic acid. Inasmuch as cytoplasmic ribonucleic acid is generally associated with active protein synthesis, it is reasonable to speculate that the synthesis of the primary keratin occurs in this zone.

The ability of tissue proteins to bind acid and basic dyes with certain selectivity at varied pH permits limited conclusions concerning their electrochemical character. In acid solutions proteins acquire a net positive charge and hence bind acid dyes. In basic solutions they acquire a net negative charge and, in consequence, bind basic dye.

In the present study the earliest fibrils demonstrable in the ordinary light microscope are shown to possess a prominently basic electrochemical character similar to that of the hardened cortex, as determined by their capacity for binding acid dyes at a high pH.

In the zone of unstabilized fibrils the hair diameter diminishes to its final value, presumably as a consequence of cellular elongation and dehydration. The disappearance of ribonucleic acid implies a cessation of active protein synthesis. In this region the birefringence increases and the presence of abundant thiol groups of protein is observed. The fibrillar structures have much the same electrochemi-

cal character as the final hair. This is the "pre-keratinous region" described by morphologists (1).

Coincident with the development of optical homogeneity and establishment of disulfide linkages, the phase described by Mercer as consolidation or progressive hardening ensues. The loss of basic dye binding capacity serves to indicate a progressive alteration distal to the unstable fibrous region and suggests that final consolidation of the hair cortex involves a loss of reactive acidic groups.

SUMMARY

Mercer has demonstrated in plucked human hair several zones of increasing molecular stability throughout the segment of hair root enclosed by the internal root sheath. These findings warranted re-examination of morphologic concepts of keratinization which indicated that keratinization was completed within the lower one-third of the hair root. In the present investigations microscopic sections of human scalp were employed to permit observation of the entire developing hair cortex.

The distribution of thiol and disulfide groups as well as ribonucleic acids are demonstrated histochemically and correlated with the birefringence of the developing cortex. The results of a study of the acid and basic dye binding capacity of fibrous structures of the forming cortex are presented. The observations are discussed in relation to chemical knowledge of the keratinization process.

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REFERENCES

1. Auber, L.: The anatomy of follicles producing wool-fibres, with special reference to keratinization. Trans. Roy. Soc. Edinburgh **LXII,** Part I (No. 7): 191–254, 1952.
2. Giroud, A. and Bulliard, H.: Croissance et keratinisation des phanères. Arch. de Zool. Exper., **75:** 583–594, 1934.
3. Mercer, E. H.: Some experiments on the orientation and hardening of keratin in the hair follicle. Biochim. et Biophys. Acta, **3:** 161–169, 1949.
4. Coons, A. H., Leduc, E. H. and Kaplan, M. H.: Localization of antigen in tissue cells. VI. The fate of injected foreign proteins in the mouse. J. Exp. Med., **93:** 173–188, 1951.
5. Barrnett, R. J.: Histochemical demonstration of protein bound sulfhydryl groups. Science, **116:** 323–327, 1952.
6. Barrnett, R. J.: Personal communication to author. 1952.
7. Deane, H. W.: The basophilic bodies in hepatic cells. Am. J. Anat., **78:** 227–243, 1946.
8. Singer, M. and Morrison, P. R.: The influence of pH, dye, and salt concentration on the dye binding of modified and unmodified fibrin. J. Biol. Chem., **175:** 133–145, 1948.
9. Schmidt, W. J.: Handb. der Biol. Arbeitsmethoden (Abderhalden). Abt. V, Teil 10, p. 588, 1938.
10. Barrnett, R. J.: The histochemical distribution of protein bound sulfhydryl groups. J. Nat. Cancer Inst., **13:** 905–925, 1953.
11. Hardy, M. H.: The histochemistry of hair follicles in the mouse. Am. J. Anat., **90:** 285–338, 1952.

DISCUSSION

DR. GEORGE F. ODLAND (in closing): Ribonucleic acids are demonstrated in the earliest phases of hair formation, namely, the fibrillation zone and the unstable fibril zone. Their presence is generally associated with active protein synthesis and in view of the fact that the fibrillar structures there formed possess many of the electrochemical characteristics of fully formed hair, one might speculate that these zones are the zones of active protein synthesis.